Applied Surface Science xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Applied Surface Science

journal homepage: www.elsevier.com/locate/apsusc

Fabrication and characterization of conductive anodic aluminum oxide substrates

Sevde Altuntas[a], Fatih Buyukserin[b,*]

[a] Micro and Nanotechnology Graduate Program, TOBB University of Economics and Technology, Ankara 06560, Turkey
[b] Department of Biomedical Engineering, TOBB University of Economics and Technology, Ankara 06560, Turkey

ARTICLE INFO

Article history:
Received 31 October 2013
Received in revised form 2 June 2014
Accepted 3 June 2014
Available online xxx

Keywords:
Biomaterials
Neural tissue engineering
Cues
Anodized aluminum oxide
Conductive surface
PVD

ABSTRACT

Biomaterials that allow the utilization of electrical, chemical and topographic cues for improved neuron–material interaction and neural regeneration hold great promise for nerve tissue engineering applications. The nature of anodic aluminum oxide (AAO) membranes intrinsically provides delicate control over topographic and chemical cues for enhanced cell interaction; however their use in nerve regeneration is still very limited. Herein, we report the fabrication and characterization of conductive AAO (CAAO) surfaces for the ultimate goal of integrating electrical cues for improved nerve tissue behavior on the nanoporous substrate material. Parafilm was used as a protecting polymer film, for the first time, in order to obtain large area (50 cm^2) free-standing AAO membranes. Carbon (C) was then deposited on the AAO surface via sputtering. Morphological characterization of the CAAO surfaces revealed that the pores remain open after the deposition process. The presence of C on the material surface and inside the nanopores was confirmed by XPS and EDX studies. Furthermore, *I–V* curves of the surface were used to extract surface resistance values and conductive AFM demonstrated that current signals can only be achieved where conductive C layer is present. Finally, novel nanoporous C films with controllable pore diameters and one dimensional (1-D) C nanostructures were obtained by the dissolution of the template AAO substrate.

1. Introduction

Development of effective biomaterials for neural tissue engineering requires the optimization of topographic, chemical and electrical cues that influence the cell–material interactions [1–4]. Significant amount of research has been conducted to utilize combinations of these cues on a rainbow of different substrates for increased cell adhesion, proliferation and alignment as well as enhanced neurite outgrowth. For instance, using biomaterials with nanoscale topography that resembles the hierarchical structure of the extracellular matrix promotes select protein adsorption/bioactivity and cell interaction [3–6]. Patterned surfaces with these features create opportunities to align the neurons which have potential to be used as neuron guidance conduits for successful neuroregeneration. Regarding chemical cues, a variety of different strategies were employed for promoting nerve regeneration. These involve the modification of substrate surface with ECM proteins or neuroactive peptides as well as doping the substrate with biochemicals such as drugs and growth factors [6,7]. Finally, studies over the past few decays have demonstrated that electrical stimulation can accelerate neural tissue regeneration. Here, bulk or nanofiber-based conducting polymeric scaffolds [5,7,8] and carbon nanotubes [9,10] are emerging as novel conductive platforms that can improve the modulation of neuronal responses.

Nanoporous anodic aluminum oxide (AAO) membranes are a unique class of biomaterials that can be synthesized by anodization of high purity aluminum [11,12]. Their intrinsic properties allow one to tune several parameters for obtaining improved tissue regeneration, and hence these biomaterials are widely used especially for bone tissue engineering applications [13,14]. For instance, the native porous structure provides a nanoscale topography upon which improved osteoblast adhesion and matrix formation was attained when compared with non-porous counterpart that does not promote osseointegration [15]. Furthermore, the pores of this

* Corresponding author. Tel.: +90 3122924513.
E-mail address: fbuyukserin@etu.edu.tr (F. Buyukserin).

http://dx.doi.org/10.1016/j.apsusc.2014.06.007
0169-4332/© 2014 Elsevier B.V. All rights reserved.

biocompatible material can be filled with chemicals or bioactive materials to promote osseointegration [11,15]. Despite the ability to manipulate such topographic and chemical cues, the research for utilizing AAO membranes for neural tissue engineering is still very limited and the related studies mainly focus on the role of topography in cell–material interaction [16–18]. Hence, exploiting the full potential of AAO as a promising substrate for neuroregeneration by utilizing the topographic, chemical and electrical cues has a great potential for neural tissue engineering applications, however, it currently still remains to be a challenge.

In this study, we focus on the fabrication and characterization of conductive AAO (CAAO) substrates to eventually control nerve tissue behavior via utilizing electrical cues. We first introduce a new approach for producing large area free-standing AAO membranes by the use of parafilm as an AAO protecting layer. We then show that CAAO surfaces with open nanopores can be prepared by carbon (C) sputtering through morphological, chemical and electrical characterization of the material. Finally, we report the formation of novel nanoporous C films and one dimensional (1-D) C nanostructures after the removal of the AAO templates.

2. Experimental details

2.1. Preparation of the free-standing AAO membranes

High purity Al foils (99.999%, Puratronic, 1 mm thickness, Alfa Aesar) were sanded with 600 grit sand paper, rinsed with deionized water (18 MΩ, Sartorius) and annealed at 450 °C for 4 h. After cooling to room temperature, an electrochemical polishing step at 15 V was applied to the foils using a Pb cathode for 90 min at 75 °C. The electropolishing solution consisted of 95 wt% H_3PO_4 (BDH Prolabo), 5 wt% H_2SO_4 (Fluka) and 20 g/ml CrO_3 (Fluka). These foils were then subjected to single or two-step anodization processes depending on the desired final nanopore size. Nanopores with ~250 nm diameter were obtained using a single step anodization where 160 V anodization potential was applied to the Al foils in a 0.4 M H_3PO_4 electrolyte at 0 °C against a stainless steel cathode. The nanopores were then widened in an aqueous chromic acid solution at room temperature for 22 min.

In order to obtain monodisperse nanopores with ~100 nm pore diameter, the well-known two-step anodization method [19,20] was followed. Here, the first anodization step was carried out at 50 V in a 5 wt% aqueous oxalic acid electrolyte solution for 18 h at 5 °C. A thick non-uniform alumina (Al_2O_3) film that forms on both sides of the Al foil was removed using an aqueous solution composed of 0.4 M H_3PO_4 and 0.2 M CrO_3 (Fluka) at 75 °C. A second anodization step was then applied at the same conditions of the first one for 5 min and the substrate was then immersed into a 5 vol% H_3PO_4 solution to widen the nanopores to yield AAO films with 100 nm ordered uniform nanopores were attained. Note that through this report, AAO structures are named as films if they are bound to the underlying Al, and membranes when they are liberated.

AAO films form on both sides of the Al foil and obtaining free-standing AAO membranes requires the removal of the Al foil that is sandwiched between these films (Fig. 1). This is typically achieved by applying a protecting polymer layer on the surface of one of the AAO films. The unprotected AAO film and the Al foil were then removed in appropriate solutions and free-standing AAO membranes were achieved after the protecting polymer layer was dissolved in an organic solvent (Fig. 1). In our case, parafilm (Bemis) was used, for the first time, as the protecting layer and the unprotected AAO film was dissolved in 1 M NaOH (BDH Prolabo) to expose the metalic Al surface. Al was then oxidized in 0.1 M $CuCl_2{\cdot}2H_2O$ (Alfa Aesar) + 6.1 M HCl (Merck) solution, and AAO

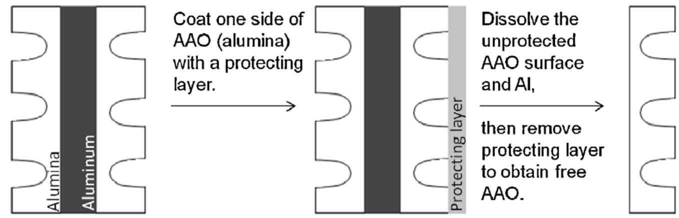

Fig. 1. Schematic representation of free-standing AAO membrane production via the application of protecting polymer layer.

membranes were obtained by dissolving the parafilm in n-hexane (Sigma–Aldrich). Nail polish (Plomar), Poly (methyl methacrylate) (PMMA, Aldrich, 350,000 MW) and lacquer (Polisan) were also used as alternative protecting layers; however, best results were obtained with parafilm as discussed in the following sections.

2.2. Preparation and characterization of CAAO surfaces

The released AAO membranes have two distinct surfaces, the barrier side and the solution side [21]. Nanopores with ~100 or 250 nm pore diameters are located on the solution side of the membranes. In order to create CAAO surfaces, the solution sides of the membranes were coated with 20-nm-thick C layer via sputtering (GATAN Presicion Etching & Coating System) unless mentioned otherwise. The C thickness value was also verified by independent ellipsometer studies which confirmed the thickness reading from the sputtering instrument with less than 10% deviation (data not shown). These CAAO surfaces were then characterized by using Scanning Electron Microscope (SEM) with an Energy Dispersive X-ray (EDX) detector (ESEM, Quanta 200 FEG), Atomic Force Microscope (AFM, EZ-AFM, tapping mode, PPP cantilever, Nanomagnetics), X-Ray photoelectron spectroscopy (XPS, K-Alpha, Thermo Scientific). The electrical characterization of the CAAO surfaces was made by measuring the surface resistance of the structures via a DC Voltage/Current Source (Yokogawa GS 210 sourcemeter). Here, CAAO surfaces were first fabricated by

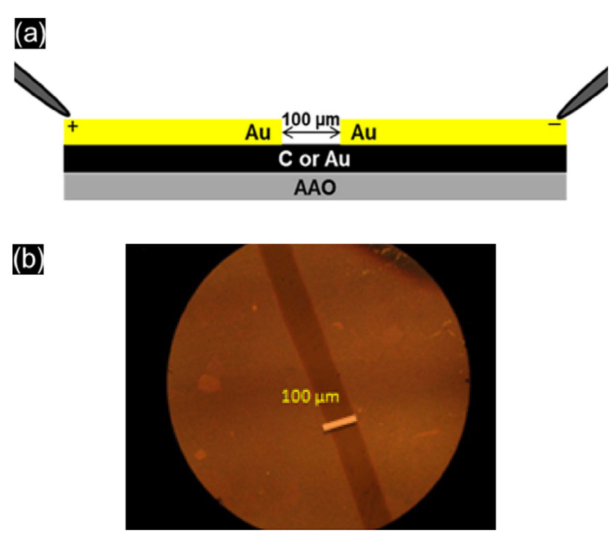

Fig. 2. Cross-sectional drawing of the masked C or Au sputtered AAO surface used in surface resistance calculation (a), and top view optical image of the masked structure showing the 100 μm-gap created by the mask.

Please cite this article in press as: S. Altuntas, F. Buyukserin, Fabrication and characterization of conductive anodic aluminum oxide substrates, Appl. Surf. Sci. (2014), http://dx.doi.org/10.1016/j.apsusc.2014.06.007